To Study and Analyze the behavior of Protein Engineering in a Recent Trends

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Abstract— Protein engineering is the conception and production of unnatural polypeptides, often through modification of amino acid sequences that are found in nature. Proteins are the principal catalytic agents and molecular machines in cells. But individual proteins do not function alone but they must interact with other molecules to carry out their cellular roles. Hence protein interfaces have become one of the most popular new targets for rational drug design. In addition to practical applications in drug design, reliable identification of protein–protein interfaces is important for basic research on the mechanisms of macromolecular recognition also for the theoretical basis. Protein engineering is the design of new enzymes or proteins with new or desirable functions. This review article describes the basic knowledge of Protein Engineering which is used in recent days.

Key words: Protein, Catalytic Agents, Cells, Drug Design, DNA Technology

I. INTRODUCTION

Reliably pinpointing which specific amino acid residues form the interface(s) between a protein and its binding partner(s) is critical for understanding the structural and physicochemical determinants of protein recognition and binding affinity, and has wide applications in modeling and validating protein interactions predicted by high throughput methods, in engineering proteins, and in prioritizing drug targets[1]. Many biochemical and/or biophysical experimental methods have been used to identify and characterize protein–protein interfaces at the level of individual atoms or residues. Widely used techniques include: X-ray crystallography [2] and nuclear magnetic resonance (NMR) spectroscopy [3], both of which are capable of determining interfaces at the atomic level; allanite scanning mutagenesis, which can determine interfaces at the residue level; various mass spectrometry-based approaches, such as chemical cross linking and hydrogen/deuterium (H/D) exchange, which typically report the location of interfaces at lower resolution, but are capable of identifying individual interfacial residues [4]; and various NMR-based approaches [5], such as chemical shift perturbations, cross-saturation, and H/D exchange, which determine interfaces at the residue or atomic level.

A. *Characteristics of Protein Interfaces*

To reliably predict interfacial residues, one needs to identify the characteristics that distinguish the interface region from the rest of the protein sequences or 3D structures. Such characteristics (or features) are critical for the success of a predictor. Widely used features in the literature include following.

- Amino acid types: The most straightforward feature is an amino acids identity or type. For classifiers that can process only numerical features, each type of commonly occurring amino acid can be represented as a binary vector of size 20 by 1.
- Physicochemical properties of amino acids: Commonly used physicochemical properties are hydro phobicity, charge and van der Waals volume. A database of numerical indices representing various physicochemical properties of amino acids and pairs of amino acids is provided in [6].
- Interface propensity: The different physicochemical properties of amino acids result in differential interaction propensities. For example, in hetero complexes, polar residues appear more frequently than do hydrophobic residues [7] and aromatic amino acids tend to form stacking interactions. The higher its interface propensity, the more likely an amino acid is to appear in the interface as opposed to elsewhere on the protein surface. Such propensities are usually derived from an analysis of known structures in the PDB.
- Evolutionary information: Interfacial residues are important functional sites and tend to be conserved among homolog [8] or undergo correlated mutations. There are different ways to encode sequence conservation, and a widely used approach is to construct PSSMs (Position Specific Scoring Matrices) from multiple sequence alignments (MSAs). Each score in a PSSM is a log-likelihood ratio of an amino acids appearance in a specific column of an MSA against a background distribution, representing the degree of conservation of the amino acid in that specific position; the higher the score, the higher the degree of conservation. Therefore, PSSMs capture important evolutionary information by exploiting the large number of available protein sequences, which are much easier to obtain than protein structures.
- Relative solvent accessibility: Most proteins recognize and interact with other proteins through their surface residues (i.e., residues with relatively high solvent accessible surface area) unless the interacting proteins undergo large conformational changes upon binding. Therefore, knowledge of protein surface residues can greatly reduce the prediction search space and increase prediction accuracy. Given the 3D structure of a protein, whether a residue is on the surface or not can be determined by calculating its relative accessible surface area.

II. LITERATURE SURVEY

Timothy M. Pabst, Ronnie Palmgren et. all [9]: They describe novel Staphylococcal Protein A ligands that enable milder elution pH for use in affinity chromatography. The change in elution pH is the result of point mutations to the

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protein sequence. Two novel ligands are investigated in this study. The first, designated Z(H18S)4, represents a histamine to serine substitution single mutation. The second, designated Z(H18S, N28A)4, is a double mutant comprising histidine to serine and asparagines to alanine mutations. Both are compared against the unmetered sequence, designated Z4, which is currently utilized in a commercially available Protein A stationary phase for the purification of molecules containing Fc domains. This work demonstrates novel Staphylococcal Protein A mutations that result in milder elution pH for use in affinity chromatography. With one exception, this was accomplished without sacrificing dynamic binding capacity or other desirable attributes such as selectivity. This work highlights the great potential Protein A still holds for future advances. As has happened in the past, improvements to this important bio processing platform technology will help drive the next generation of protein therapeutics.

Pete Gagnon and Rui Nian [10]: In this work Size and conformation of an IgG1 monoclonal antibody eluted from protein A were influenced by protein A-mediated denaturation of IgG, by IgG concentration, by pH and conductivity. IgG on the leading side of the elution profile exhibited a hydrodynamic diameter of 9.4 nm and relatively minor disruption of secondary structure. Size diminished across the main body of the peak to a minimum of2.2 nm with a highly non-native secondary structure. Size reduction dominantly derived from a pre-existing tendency of IgG to adopt smaller conformations at elevated concentrations, low pH and conductivity. Conformational disruption of the antibody upon initial contact with protein A facilitated the transition and enabled it to occur at modest IgG concentrations. Dramatic loss of secondary structure across tailing fractions was accompanied by an increase in IgG size up to 10.4 nm, apparently caused by excess helix extending the hydrodynamic axis of the protein.

Haibin Luoa, Nacole Lee et all [11]: In this work, they propose a new mechanism that may explain some of these observations. Through a systematic evaluation of three different mAbs, they determined that this transient turbidity is most likely caused by LLPS. The dense phase liquid droplets consist of high concentration mAb proteins and are also highly viscous, resulting in high column back pressure during the elution process. Lower load challenge, higher operating temperature, lower elution flow rate, higher sodium chloride concentration in the elution buffer, addition of argentine in the elution buffer, increasing elution buffer concentration, or use of a mildly acidic wash buffer preceding elution are all viable options demonstrated in this work to control turbidity and column back pressure. Their results revealed that the use of a mildly acidic buffer preceding elution is the most suitable solution for reducing the elution turbidity in large-scale. Their results also suggest that it is likely LLPS occurs frequently during Protein A chromatography in mAb downstream processing. The under-reporting in the literature is possibly due to the transient and reversible nature of LLPS.

Alice R. Mazzera, Luke A. Clifton et all [12]: In the first part of the work, we showed that IgG4 adsorbs directly to silica at a high packing density in a largely flat-on orientation at pH 4.1. While the IgG solution remained in the cellar fraction of weakly adsorbed material was also observed; data suggested partial re-orientation of this material into the flat on-configuration on rinsing the surface with buffer. The data demonstrated that electrostatic attraction between IgG and surface was a strong driving force for surface adsorption, and protein-protein repulsion was a factor that influenced the packing density of the interfacial layer. In the second part of the work model surface including recombinant staphylococcal protein Across-linked to silica, and one of two blocking molecules, BSA orPEG6000, was examined by FTIR and neutron reflectivity. The FTIR data indicated that rSPA was successfully cross-linked to the surface and retained its structure for IgG binding. IgG was found to elute at a higher pH than is typically expected. FTIR data also demonstrated a lack of non-specific binding to the model surface.

K.E. Preece a, N. Hooshyar et all [13]: Ultrasound treatment was shown to significantly improve the protein extraction yield by 4.2% during the okara solution treatment on pilot plant scale. Okara solution flow rate and okara concentration also had significant effects on the protein extraction yield. However, considering the whole soy base production process, from soybeans to final processing materials studied, UAE was found to have comparable results to the washing of okara at pilot-scale, contrary to lab-scale sanitation. During the lab-scale sanitation treatment, greater energy intensity was experienced by the samples compared to the pilot-scale system, resulting in a greater impact of ultrasound treatment. Okara solution visualised after pilot-scale sanitation was found to still contain intact cells, complete with protein bodies inside. For the extraction of soy protein, one of the world's cheapest and most readily available protein sources, ultrasound is not considered to be the most beneficial unit operation for enhancing the extraction yield, for reasons including the life of the probe and the high energy input.

Alan S. Campbell, Hironobu Murata et all [14]: We have developed and thoroughly characterized a GOX-based electrode system formed by the growth of poly(N-(3-dimethyl (ferrocenyl) methy l ammonium bromide) propyl acryl amide)from the enzyme surface via PBPE techniques followed by the physical adsorption of these GOX-pFc Conjugates onto gold/MWCNT fibber paddle electrodes. The final GOX-pFcAc-gold/MWCNT fibre paddles anodes proved capable of MET through the covalently attached red ox polymer chains while maintaining GOX bio catalytic activity. The effective "wiring" of GOXthrough pFcAcledtoa24- fold increase in current generation efficiency compared to native GOX adsorbed onto the same electrode material. This performance enhancement extended to the capability of GOX pFcAc-gold/ MWCNT fibre paddle anodes coupled with BOD-gold/MWCNT fibre paddlecathodestoproducea4-foldgreaterEBFCpower density (1.7 mW cm2) compared to GOX-gold/MWCNT fibre paddle anodes wit out the presence of free mediator and thus no need for compartmentalization.

Ian Sillitoe, Natalie Dawson et. all [15]: This article presents a historical review of the protein structure classification database CATH. Together with the SCOP database, CATH remains comprehensive and reasonably up-to-date with the now more than 100,000 protein structures in the PDB. They review the expansion of the CATH and SCOP resources to capture predicted domain structures in

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the genome sequence data and to provide information on the likely functions of proteins mediated by their constituent domains. The SCOP and CATH classifications organize the 3D structure of proteins into evolutionary classifications that have enabled detailed studies of the molecular mechanisms by which new protein structures and functions evolve. The sequence patterns and fold libraries that they provide have enabled prediction of structural relatives thereby providing structural annotations for more than 50 million domain sequences, available on their sister sites. The predicted data revealed the power law bias in super family populations whereby most super families are small but a few hundred are universal and very highly populated.

C. Safi, G. Olivieri et all [16]:- A mild bio refinery process was investigated on the microalgae Nannochloropsis Guadiana, to obtain an enriched fraction of water soluble proteins free from chlorophyll. After harvesting, a 100 g.L 1 solution of cells was first subjected to cell disruption by either high-pressure homogenization (HPH) or enzymatic treatment (ENZ). The main objective of this study was to carry out a mild bio refinery process with concentrated microalgae to obtain a protein fraction in the filtrate after testing two cell disruption methods. The results demonstrated that homogenization was more efficient than the protease treatment in terms of cell disintegration and release of soluble proteins. However, the filtration process revealed higher efficiency for the protease treated samples with a 25% protein yield obtained in the filtrate of the 300 kDa membrane. The study also concluded that increasing the cut off of the membrane does not necessarily improve the performance of the process, especially for native proteins.

Jennifer Konczal and Christopher H. Gray [17]:- Protein production facilities are often required to produce diverse arrays of proteins for demanding methodologies including crystallography, NMR, ITC and other reagent intensive techniques. It is common for these teams to find themselves a bottleneck in the pipeline of ambitious projects. This pressure to deliver has resulted in the evolution of many novel methods to increase capacity and throughput at all stages in the pipeline for generation of recombinant proteins. This review aims to describe current and emerging options to accelerate the success of protein production in Escherichia coli. We emphasize technologies that have been evaluated and implemented in our laboratory, including innovative molecular biology and expression vectors, small-scale expression screening strategies and the automation of parallel and multidimensional chromatography. Improved screening increases the number of soluble, highly expressing systems that progress to scale up, putting pressure on preparative scale purification.

David J. Huggins [18]:- In this paper they employ molecular dynamics simulations to address the first of these questions. Computational methods are well-placed to address this issue due to their ability to analyze systems at atomic-level resolution. Traditionally, the stability of folded proteins has been ascribed to the balance of two types of intermolecular interactions: hydrogen-bonding interactions and hydrophobic contacts. In this study, they explore a third type of intermolecular interaction: cooperative hydration of protein surface residues. To achieve this, they consider multiple independent simulations of the villain headpiece domain to quantify the contributions of different interactions to the energy of the native and fully extended states. In addition, they consider whether these findings are robust with respect to the protein force field, the water model, and the presence of salt. In all cases, they identify many cooperatively hydrated interactions that are transient but energetically favor the native state. The results of this work highlight a potential role for cooperative hydration as a driving force in protein folding and suggest a number of villain mutations that can be used to test the prediction. Whilst this is not a definitive article on cooperative hydration or protein folding, it suggests that we should explicitly consider the importance of salvation effects in protein folding. They suggest that support from additional force fields, water models, and protein test cases are needed to establish this as a general design principle.

III. CONCLUSION

The modification of natural enzymes and proteins by protein engineering is an increasingly important scientific field. The well-known methods of rational design and directed evolution, as well as new techniques will enable efficient and easy modification of proteins. New technologies such as computational design, catalytic antibodies and mRNA display would be crucial for de novo engineering of enzymes and also for new areas of protein engineering. Protein engineering applications cover a broad range, including bio catalysis for food and industry, as well as medical, environmental and nano biotechnological applications. With advances in recombinant DNA technology tools, “omics” technologies and high-through put screening facilities, improved methods for protein engineering will be available, which would enable easy modification or improvement of more proteins/enzymes for further specific applications.

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